

The discovery of Szent-Györgyi and co-workers² that citrus fruits contain a substance not identical with hesperidin or any other identified constituent of these fruits has led to much chemical and physiological research in an endeavor to isolate and characterize the active compound, which has been termed vitamin P.³ Investigations begun in 1943 have established the fact that rutin, a flavonol glucoside present in some forty species of plants, has the physiological activity attributed to vitamin P.⁴ The question whether the activity reported from citrus fruits which may

be due to the presence of rutin has been investigated in this Laboratory. Valencia oranges, grapefruit and both green and ripe lemons, reported to be most active of the citrus fruits with respect to vitamin P activity, have been examined without revealing the presence of rutin. These have been subjected to three extraction procedures: the usual alcoholic technique,⁵ a special process involving enzymatic hydrolysis of pectin, and an alkali extraction. In no case was rutin found by the alcoholic extraction. In the instance of a hybrid fruit, rutin was found by the other two processes, the alkali extraction described below being more effective.

Two years ago we obtained from the orchard of A. C. Krewson, Plymouth, Florida, specimens of a citrus fruit of the size and form of grapefruit but with a distinct odor and flavor of the lemon. The

(1) One of the Laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture. Article not copyrighted.

(2) P. L. Armentano, *et al.*, *Deut. Med. Woch.*, 62, 1326-1328 (1936).

(3) V. Bruckner and A. Szent-Györgyi, *Nature*, 138, 1057 (1936).

(4) J. Q. Griffith, Jr., J. F. Couch and M. A. Lindauer, *Proc. Soc. Exptl. Biol. Med.*, 55, 228-229 (1944); J. F. Couch, C. F. Krewson, J. Naghaki and M. J. Copley, U. S. Department of Agriculture, Mimeograph Circular AIC-115, April, 1946.

(5) J. F. Couch and C. F. Krewson, U. S. Department of Agriculture Mimeograph Circular AIC-52, July, 1944.

juice of these fruits served to make excellent "lemonade." The hybrid has been tentatively identified by F. E. Gardner of the Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, as a cross between grapefruit and Satsuma orange, produced during experiments on cross-breeding citrus fruits and named Satsumelo.

Examination of the peel of these fruits finally resulted in the isolation of significant quantities of rutin after the usual alcoholic extraction had been unsuccessful. Exhaustive maceration with alkalis extracted the constituent slowly. Rind of green fruits picked January 3, 1946, gave 3.2% of rutin (moisture-free basis), whereas the rind of ripe fruit picked the previous year, February 10, 1945, gave approximately 0.9%, and that of over-ripe fruit picked April 28, 1945, yielded about 0.1% of rutin.

These data are consistent with the facts obtained with other rutiniferous plants⁶ that immature tissues, plants or fruits contain more rutin than more mature tissues. Rutin appears to be associated with the most actively metabolizing portions of the plant, a fact in harmony with Szent-Györgyi's view that the flavonols form part of the oxidation-reduction chain in the plant cell.⁷

No trace of rutin was found, in repeated attempts both by alcoholic and alkali extraction techniques, in the peeled fruit, the pressed juice of the peeled fruit, the pulp of the peeled fruit, the fermented whole fruit, or in the air-dried leaves of the tree from which the fruits were obtained.

Experimental

The minced peel of the hybrid (258.5 g.) was mixed with 757 ml. of distilled water and sufficient 3 *N* sodium hydroxide solution to bring the mixture to a pH of 11, and stirred for one hour. The residue after filtration was again macerated with sodium hydroxide solution for one hour and filtered. This treatment was repeated twice

(6) J. F. Couch, J. Naghski and C. F. Krewson, *Science*, 103, 197-198 (1946).

(7) S. Szent-Györgyi, "Studies on Biological Oxidation and Some of Its Catalysts," Barth, Leipzig, 1937, pp. 67-98.

more. The filtrates were separately adjusted to pH 4.1 with hydrochloric acid and allowed to stand in a refrigerator. The first two extracts yielded 1.50 and 0.36 g. of rutin, respectively, a total of 1.86 g. or 3.23% of the moisture-free material. The substance recrystallized from boiling water gave a plastic range ("melting point") of 185.5 to 187.5° (cor.). Spectrophotometric examination of the material after recrystallization from absolute ethanol revealed absorption maxima at wave lengths 2575 and 3625 Å. with a specific extinction coefficient of 32.74 at 3625 Å. Purified rutin from buckwheat, after recrystallization from absolute ethanol, shows maxima at the same wave lengths, with a coefficient of 32.55 at 3625 Å.⁸

A portion of the rutin was further purified by solution in alcohol and recrystallization from water.

*Anal.*⁹ Calcd. for $C_{27}H_{30}O_{16}$: C, 53.11; H, 4.95. Found: C, 53.07; H, 5.11.

Hydrolysis of Rutin.—The purified glucoside (0.200 g.) was heated one hour with 8 ml. of 5% sulfuric acid and cooled overnight. The precipitated quercetin was filtered, washed and recrystallized from ethanol. When it was dried at 110° it melted at 313-313.5°.

*Anal.*⁹ Calcd. for $C_{15}H_{10}O_6$: C, 59.61; H, 3.34. Found: C, 59.57; H, 3.45.

The pentaacetate melted at 194-196° and showed no depression when mixed with authentic quercetin pentaacetate. The carbohydrates produced by hydrolysis of the rutin were identified as rhamnose and glucose by the usual methods.

Summary

Rutin has been isolated from the rind of Satsumelo, a hybrid citrus fruit resulting from a cross between grapefruit and Satsuma orange.

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(8) The specific extinction coefficient of purified rutin from buckwheat previously reported (*THIS JOURNAL*, 69, 572-573 (1947)), 31.9 liter g.⁻¹ cm.⁻¹, is raised to 33.22 by addition of a small quantity of acetic acid to the rutin solution prepared for spectrophotometric examination. The addition of this acid increases the coefficient but renders it insensitive to accidental traces of alkali present on the glassware or in the solvent. See W. L. Porter, B. A. Brice, M. J. Copley and J. F. Couch, U. S. Department of Agriculture Mimeograph Circular AIC-159, July, 1946, "Tentative Spectrophotometric Method for the Determination of Rutin in Various Preparations." Recrystallization of the purified buckwheat rutin from absolute ethanol, followed by application of the modified procedure, results in a specific extinction coefficient of 32.55 at 3625 Å., with an unchanged extinction ratio of 0.875 \pm 0.004 for wave lengths 3750 and 3625 Å. We are indebted to W. L. Porter for the spectrophotometric work.

(9) Analyses by C. L. Ogg of this Laboratory.